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# Effects of cover crops, compost, and manure amendments on soil microbial community structure in tomato production systems

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## ABSTRACT

Soil microbial community structure and crop yield was investigated in field tomato production systems that compared black polyethylene mulch to hairy vetch mulch and inorganic N to organic N. The following hypotheses were tested: (1) hairy vetch cover cropping increases crop yield and significantly affects soil microbial community structure when compared to the standard plastic mulch and synthetic fertilizer-based system; (2) within plastic mulch systems, organic amendments will increase crop yield and significantly affect soil microbial community structure when compared to synthetic fertilizer; (3) crop yields and microbial community structure will be similar in the hairy vetch cover cropping and the organic amended plasticulture systems. Treatments consisted of ammonium nitrate (control), hairy vetch cover crop, hairy vetch cover crop and poultry manure compost (10 Mg/ha), three levels of poultry manure compost (5, 10, and 20 Mg/ha), and two levels of poultry manure (2.5 and 5 Mg/ha). Black polyethylene mulch was used in all treatments without hairy vetch. Fatty acid analysis was used to characterize the total soil microbial community structure, while two substrate utilization assays were used to investigate the community structure of culturable bacteria and fungi. Crop yield was not significantly increased by hairy vetch cover cropping when compared to black polyethylene mulch, although microbial community structure was significantly affected by cover cropping. Under black polyethylene mulch, crop yields were significantly increased by the highest levels of compost and manure when compared to inorganic fertilizer, but there was no detectable effect on soil microbial community structure. When cover cropping was compared to organic amended plasticulture systems, crop yields were similar one year but dissimilar the next. However, hairy vetch cover cropping and organic amendments under black plastic mulch produced significantly different soil microbial community structure.

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## 1. Introduction

Conventional tomato production utilizing tillage, black plastic mulch, commercial fertilizer applied through drip irrigation,

and pesticides, can improve the yield and quality of fresh-market tomatoes compared to bare soil production by warming soil earlier in spring, preventing evaporation of soil moisture, increasing fertilizer use efficiency, and suppressing

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weeds (Abdul-Baki et al., 1992). However, this intensive production system also can degrade soil quality, enhance runoff by covering the soil with an impervious surface, contribute to surface and groundwater pollution, and add to production cost (Rice et al., 2001). Alternative systems have been developed that use renewable organic resources and/or minimize tillage to build soil organic matter and improve soil quality. Minimum-tillage systems including cover cropping with legumes to fix nitrogen, recycle nutrients, and improve soil quality have been identified as viable alternatives for production of both agronomic and horticultural crops (Decker et al., 1994; Lu et al., 2000) and have distinct economic and environmental advantages over use of plasticulture or bare soil (Kelly et al., 1995; Rice et al., 2001).

In a system developed at the USDA Beltsville Agricultural Research Center (Abdul-Baki and Teasdale, 1997), raised beds are formed in the fall and seeded with hairy vetch. The vetch is mowed the following spring just before tomatoes are transplanted through the vetch residue without tillage. The hairy vetch mulch suppresses many annual weeds and provides much of the nitrogen needed by the tomato plants. This surface mulch also reduces raindrop impact on the soil which increases infiltration, reduces runoff and sediment losses from fields, and reduces splashing that can enhance development of foliar diseases (Mills et al., 2002). While tomatoes grown in black plastic mulch produce greater early plant growth and higher early yields, tomatoes grown with hairy vetch mulch have greater overall leaf area, higher total yields, and a longer season (Teasdale and Abdul-Baki, 1997).

Manure and compost are organic sources of nutrients that also have been shown to increase soil organic matter and improve soil quality (Wright et al., 1998). They usually need to be incorporated into soils to prevent loss of nutrients through volatilization or runoff, particularly nitrogen. Thus, they would be suitable for use in conjunction with a traditional tillage-based plasticulture system but could offer potential soil quality improvement in this otherwise soil-destructive system. This approach would provide an easier alternative system for growers to adopt than the minimum-tillage cover crop system that requires new equipment and additional fall operations.

Microbial communities perform essential ecosystem services, including nutrient cycling, pathogen suppression, stabilization of soil aggregates, and degradation of xenobiotics. Soil microbial biomass, activity, and community structure have been shown to respond to agricultural management systems. Soil microbial communities can reflect the impact of management on soil quality although the linkage between soil microbial communities and soil quality is not well understood. Since many soil properties associated with soil quality do not change rapidly in response to management, we evaluated soil microbial communities which would be expected to respond more rapidly to alternative management systems in a short study such as the 2-year experiment presented in this paper (Martini et al., 2004). In this study, we compare soil microbial communities in conventional plasticulture tomato production systems with and without organic soil amendments and in the alternative cover crop-based system. The following hypotheses were tested: (1) hairy vetch cover cropping increases crop yield and significantly affects soil microbial community

structure when compared to the standard plastic mulch and synthetic fertilizer-based system; (2) within plastic mulch systems, organic amendments will increase crop yield and significantly affect soil microbial community structure when compared to synthetic fertilizer; (3) crop yields and microbial community structure will be similar in the hairy vetch cover cropping and the organic amended plasticulture systems.

## 2. Materials and methods

### 2.1. Field experiment

The experiment consisted of eight soil amendment systems for growing fresh-market tomatoes conducted on Downer-Ingleside loamy sand on the North Farm of the Beltsville Agricultural Research Center, Beltsville, Maryland, during the summer seasons of 2000 and 2001. The treatments were synthetic N only (control), three levels of poultry manure compost (5, 10, and 20 Mg/ha), two levels of poultry manure (2.5 and 5 Mg/ha), hairy vetch mulch, and (in 2001 only) hairy vetch mulch plus poultry manure compost (10 Mg/ha).

Poultry manure compost was produced at the Beltsville Agricultural Research Center Composting Facility. Compost used in 2000 consisted of poultry (turkey) litter mixed with orchard grass hay in a 1:3 (v/v) mixture, placed in a 50 m windrow and composted by the windrow method for 8 weeks. The final pH was 7.54, total organic carbon was 266 g/kg, total nitrogen was 14.9 g/kg, and total phosphorus was 15.7 g/kg (Sikora and Enkiri, 2003). Compost used in 2001 consisted of (by volume) 15% old hay, 15% mixed manure and bedding (from layers, 2–3 weeks old, 120 °C), 25% poultry (layers) manure, 30% leaves, and 15% straw. After composting by the windrow method for 9 weeks, the final pH was 8.89, total organic carbon was 74.8 g/kg, total nitrogen was 6.6 g/kg, and total phosphorus was 0.77 mg/kg.

Field preparation began in September of 1999 with the creation of raised beds, 1.5 m center-to-center and 15 cm high. Treatments were applied to plots consisting of four 12-m sections of beds and were arranged in a randomized complete block design with four replications. Hairy vetch seed (*Vicia villosa* Roth) was planted at 45 kg/ha on the top of designated beds on 10/16/99 and 10/4/00. The compost associated with the compost plus vetch treatment was applied before forming beds and planting vetch in October of 2000. The other compost and manure amendments were incorporated into designated beds and the beds reshaped on 5/8/00 and 5/8/01. Treatments were continued in the same plots in the same field in the second season.

In preparation for planting, hairy vetch was mowed and black polyethylene mulch was laid on all treatments except those with a surface mulch of hairy vetch residue. Drip irrigation tape was installed under black plastic mulch and on top of hairy vetch residue approximately 10 cm from the tomato row. 'Sunbeam' tomato seedlings (*Lycopersicon esculentum* Mill.) were transplanted into the center of each bed spaced approximately 50 cm apart on 5/18/00 and 5/16/01. Plants were irrigated throughout the season as needed. Fertilizer was injected every other week through the irrigation system. Nitrogen was applied as ammonium nitrate to provide

a seasonal total of 168 kg/ha to the control treatment. Half that rate of N was applied to the hairy vetch treatments per recommendations based on previous research which showed that vetch provides maximum yield with 89 kg/ha of fertilizer N (Abdul-Baki et al., 1997). The seasonal mineralized N contribution by the three compost treatments was estimated as 6, 11, and 22 kg/ha and that by the two manure rates was estimated as 28 and 56 kg/ha. Fertilizer N applications to the compost and manure treatments were reduced by the estimated mineralized N compared to that applied to the control treatment, thus keeping total mineralized N inputs roughly constant in all of the black polyethylene treatments. Herbicide was applied for weed control to the bare soil between rows of plastic mulch or to the entire bed area of the vetch plots. Fungicides were applied regularly after fruit set based on weekly scouting reports (5 applications in 2000 and 8 applications in 2001). Fruit was harvested every 3–5 days from the center 16 plants of the center two rows from early August to early September.

Bulk soil was sampled during the 2001 growing season on June 18, July 17, August 15, and September 6. Six 4-cm diameter soil cores were taken to a 15-cm depth along the tomato row in the center beds of each plot, composited, and placed in a cooler for transport to the lab.

## 2.2. Soil microbial community analysis

Bacterial substrate utilization was assayed using Biolog GN plates (Biolog, Inc., Hayward, CA, USA) as described previously (Buyer et al., 1999, 2002). Briefly, fresh soil samples were serially diluted to  $10^{-4}$  in sterile saline and 150  $\mu$ l were added to each well. Fungal substrate utilization was assayed using Biolog SF-N plates as described previously (Buyer et al., 2001, 2002). Briefly, soil samples were serially diluted to  $10^{-3}$  in dilute agar with antibiotics and 100  $\mu$ l were added to each well. All plates were incubated at 22 °C for 7–10 days and read daily on a microplate reader (GN plates, 595 nm; SF-N plates, 650 nm).

Fatty acids from soil lipids were directly transesterified to fatty acid methyl esters (FAME) as described previously (Gagliardi et al., 2001; Buyer et al., 2002). Briefly, frozen soil samples were lyophilized and then treated with alkaline methanol for 1 h at 37 °C. The resulting FAMES were extracted and analyzed by gas chromatography on an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with the methyl esters from 9 to 30 carbons long identified and quantified using the MIDI eukaryotic method (Microbial ID, Inc., Newark, DE, USA).

## 2.3. Statistical analysis

All data analysis was accomplished using SAS software (SAS Institute, Cary, NC, USA). ANOVA was performed on tomato yield data using the mixed model procedure (proc MIXED) with treatment as fixed effect and block as random effect. The substrate utilization assay data were analyzed after the substrates were divided into six groups and the average absorbance per category was calculated (Zak et al., 1994). The blank was used as a covariate (Buyer et al., 1999). The FAME data was analyzed after the FAMES were divided into chemical

and biomarker groups and the areas of all the peaks within each group were summed (Buyer et al., 1999, 2002). The fatty acids used for biomarkers were as follows: Gram-positive bacteria, iso and anteiso branched fatty acids; Gram-negative bacteria, monounsaturated fatty acids; fungi, 18:2  $\omega$ 6c; actinomycetes, 10 methyl 18:0; mycorrhiza, 16:1  $\omega$ 5c; protozoa, 20:3  $\omega$ 6c and 20:4  $\omega$ 6c (Buyer et al., 2002; Blackwood and Buyer, 2004). Block effects were eliminated using a mixed model (proc MIXED). Significance testing was accomplished using ANOVA and MANOVA (proc GLM) with sampling date and treatment as main effects. In a separate analysis, MANOVA results were represented graphically using canonical variates analysis (Seber, 1984; Buyer et al., 1999). For the canonical variates analysis, a one-way model was used with the main effect consisting of each combination of date and treatment. The Hellinger transformation was used to eliminate effects of inoculum density (Biolog) and biomass (FAME) by taking the grouped variables, normalizing them, and taking the square root (Eq. (1)).

$$H_i = \sqrt{\frac{F_i}{\sum_{i=1}^n F_i}} \quad (1)$$

Here  $F_i$  is the  $i$ th fatty acid group,  $n$  is the total number of groups of fatty acids, and  $H_i$  is the Hellinger-transformed fatty acid group. The equation for Biolog data is analogous. This transformation is recommended for analysis of species abundance data (Legendre and Gallagher, 2001) and has been used previously for PLFA data (Blackwood and Buyer, 2004). Data was analyzed both before and after transformation. The influence of inoculum density and biomass on the canonical variates, and the effectiveness of the Hellinger transformation at removing these effects, was tested by measuring the correlation coefficient between each canonical variate and the average absorbance of all the wells in the Biolog plate (AWCD, average well color development) or the total area of the fatty acid methyl ester peaks.

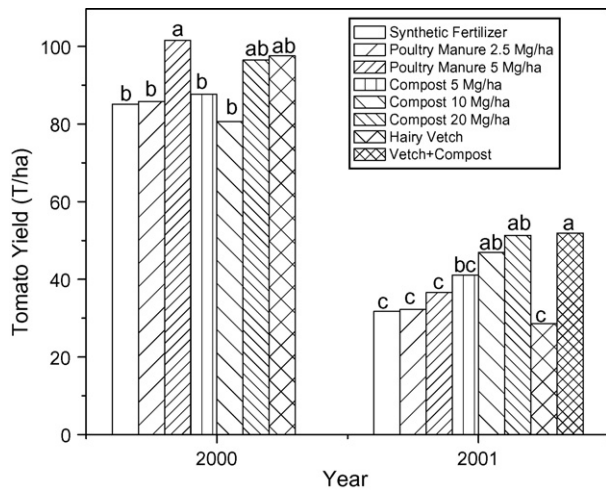
## 3. Results

### 3.1. Tomato yields

In 2000, tomato yields were significantly higher with the highest level of poultry manure than the fertilizer control (Fig. 1). The yields from compost at the highest rate and the hairy vetch treatment were similar to yield of manure at the high rate. In 2001, yields were lower than in 2000 (Fig. 1), probably because of higher foliar disease pressure. Excessive rainfall during the month of July (154 mm) provided conditions highly conducive to development of foliar diseases and led to early defoliation of plants in all treatments. The two highest levels of compost and the hairy vetch plus compost treatment had significantly higher yields than the fertilizer control.

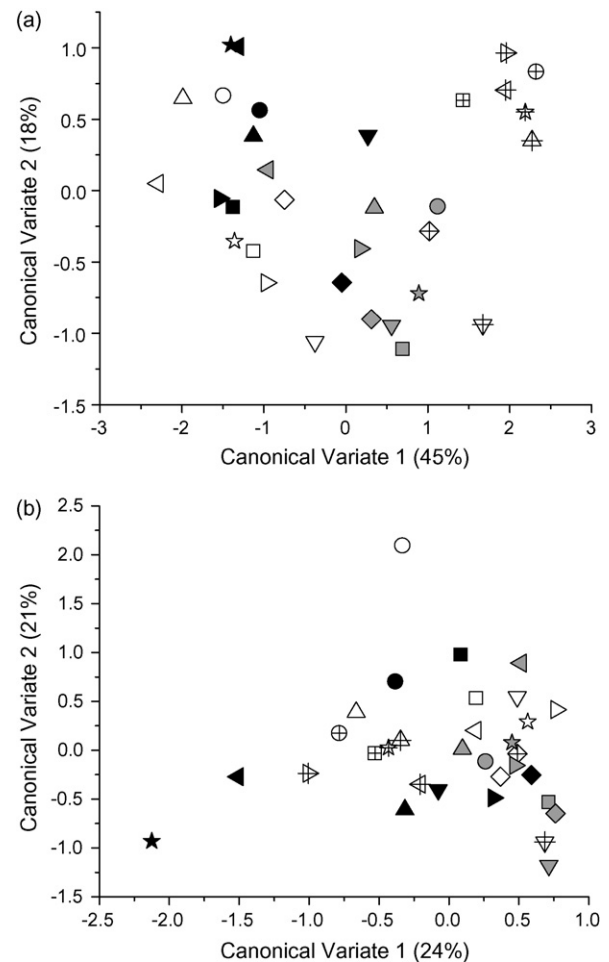
### 3.2. Bacterial substrate utilization assay

The substrate utilization assay on Biolog GN plates was used to study the community structure of rapidly growing, culturable, aerobic, heterotrophic bacteria. Readings from the fifth day of



**Fig. 1 – Tomato fruit yields for 2000 and 2001. For each year different letters indicate statistically significant differences ( $P \leq 0.05$ ).**

incubation were found to be most significant and are reported here. MANOVA results are presented in Table 1. Sampling date was generally more significant than treatment. The canonical variates are graphed in Fig. 2a for the analysis before normalization and in Fig. 2b for the analysis after the Hellinger transformation. The date effect was very clear in Fig. 2a, with the first two sampling dates on the left side of the graph, the August sampling in the center, and the final sampling to the right. The first canonical variate (X-axis) was strongly correlated ( $r = 0.94$ ) to the AWCD, indicating that the separations along the X-axis are due to differences in overall absorbance. The treatment effect was much more subtle in Fig. 2a, but the compost plus vetch and vetch treatments tended to be found in the lower center of the graph. After Hellinger transformation, both canonical variates were not correlated with AWCD. The date effect largely disappeared, as shown in Fig. 2b. There was, however, some treatment effect, with the compost plus vetch and the vetch treatments localized in the lower right corner of the graph.



**Fig. 2 – Canonical variates for bacterial substrate utilization assay: (a) before Hellinger transformation and (b) after transformation. Means for each sampling date  $\times$  treatment combination are plotted. Black symbols, 6/18/01; open symbols, 7/17/01; gray symbols, 8/15/01; crossed symbols, 9/06/01. (■) compost 5 Mg/ha; (●) compost 10 Mg/ha; (▲) compost 20 Mg/ha; (◀) poultry manure 2.5 Mg/ha; (▶) poultry manure 5 Mg/ha; (★) fertilizer; (◆) hairy vetch; (▼) vetch plus compost 10 Mg/ha.**

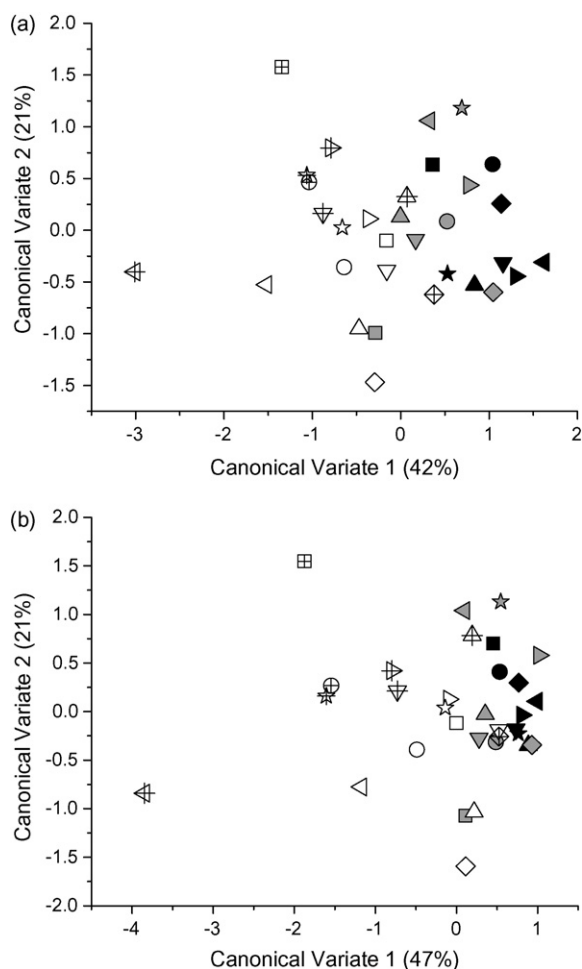
**Table 1 – Significance testing results for substrate utilization analysis of soil bacteria and fungi**

Group	Bacteria				Fungi			
	Before transformation		After transformation		Before transformation		After transformation	
	Date <sup>a</sup>	Treatment <sup>b</sup>	Date	Treatment	Date	Treatment	Date	Treatment
Polymers	HS	S	NS	NS	S	NS	NS	NS
Carbohydrates	HS	NS	S	NS	HS	NS	HS	S
Carboxylic acids	HS	NS	NS	NS	HS	NS	NS	NS
Amines and amides	HS	NS	S	S	S	NS	HS	NS
Amino acids	HS	NS	S	NS	NS	NS	S	NS
Miscellaneous	HS	NS	NS	NS	NS	NS	S	NS
Overall	HS	NS	HS	NS	HS	NS	HS	NS

<sup>a</sup> HS, highly significant ( $P \leq 0.001$ ); S, significant ( $P \leq 0.05$ ); NS, not significant ( $P > 0.05$ ).

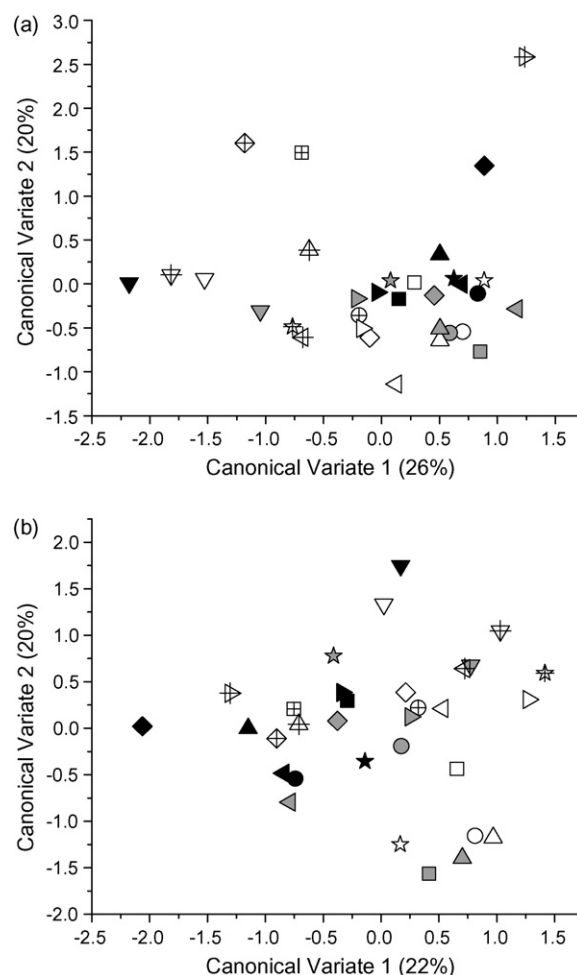
<sup>b</sup> All date  $\times$  treatment interactions were not significant.





**Fig. 3 – Canonical variates for fungal substrate utilization assay: (a) before Hellinger transformation and (b) after transformation. Means for each sampling date  $\times$  treatment combination are plotted. Black symbols, 6/18/01; open symbols, 7/17/01; gray symbols, 8/15/01; crossed symbols, 9/06/01. (■) compost 5 Mg/ha; (●) compost 10 Mg/ha; (▲) compost 20 Mg/ha; (◄) poultry manure 2.5 Mg/ha; (►) poultry manure 5 Mg/ha; (★) fertilizer; (◆) hairy vetch; (▼) vetch plus compost 10 Mg/ha.**

There was an apparent contradiction between the MANOVA using treatment as the main effect in the model (Table 1) and the canonical variates analysis, which is based on a MANOVA using a combination of date and treatment as the main effect in the model (Fig. 2). While no statistically significant treatment effect was found when tested for directly (Table 1), a treatment effect is visible in Fig. 2. We tested the significance of this apparent treatment effect by running ANOVA on the canonical variates. Before normalization (Fig. 2a), canonical variate 1 had no statistically significant treatment effect, but there was a statistically significant treatment effect for canonical variate 2 ( $P = 0.0087$ ). After normalization (Fig. 2b), both canonical variates 1 and 2 were weakly significant for treatment (canonical variate 1,  $P = 0.0423$ ; canonical variate 2,  $P = 0.0417$ ). We conclude from



**Fig. 4 – Canonical variates for fatty acid chemical groups: (a) before Hellinger transformation and (b) after transformation. Means for each sampling date  $\times$  treatment combination are plotted. Black symbols, 6/18/01; open symbols, 7/17/01; gray symbols, 8/15/01; crossed symbols, 9/06/01. (■) compost 5 Mg/ha; (●) compost 10 Mg/ha; (▲) compost 20 Mg/ha; (◄) poultry manure 2.5 Mg/ha; (►) poultry manure 5 Mg/ha; (★) fertilizer; (◆) hairy vetch; (▼) vetch plus compost 10 Mg/ha.**

this that there is a weak treatment effect on the substrate utilization data.

### 3.3. Fungal substrate utilization assay

The substrate utilization assay on Biolog SF-N plates was used to study the community structure of fungi. Readings from the seventh day of incubation were found to be most significant and are reported here. MANOVA results are presented in Table 1. Overall sampling date was highly significant both before and after normalization, but fewer substrate groups were significant than in the assay for bacteria. Treatment and date  $\times$  treatment interactions were not significant. The results from the canonical variates analyses are graphed in Fig. 3a and b. The date effect is readily seen but no treatment effect is

apparent. These two graphs are very similar to each other, indicating that the Hellinger transformation had very little effect on the canonical variates. Before transformation, canonical variate 1 was significantly correlated to AWCD ( $r = 0.68$ ), while after transformation the correlation coefficient was smaller but still statistically significant ( $r = 0.42$ ).

### 3.4. Fatty acid analysis

Fatty acid analysis was used to characterize the total soil microbial community. The FAMES were first divided into nine categories according to the chemical structure of the fatty acids. As shown in Table 2, both date and treatment were significant for several of the groups and the overall MANOVA, but no significant interactions were found. The overall effects were significant both before and after transformation, indicating that the effects were at least in part due to changes in community composition and not entirely due to changes in biomass. The only canonical variate with statistically significant correlation to total fatty acid was canonical variate 1 before Hellinger transformation ( $r = 0.49$ ). The canonical variates analysis of the data before transformation (Fig. 4a) and after transformation (Fig. 4b) clearly showed a treatment effect, with the compost plus vetch treatment clustered in each graph. There was also a date effect with the samples from 9/6/01 tending to separate from the other sampling dates.

FAMES were next divided into biomarker categories (Table 2). Both date and treatment effects were statistically significant. While only date affected Gram-negatives, actinomycetes, and mycorrhizal biomarkers, fungal and protozoan biomarkers were affected by both date and treatment, with treatment effects both before and after transformation. The canonical variates analysis before transformation was not statistically significant. The compost plus vetch and hairy

vetch treatments were distinguishable from all other treatments in the canonical variates analysis after transformation (Fig. 5). The canonical variates were not significantly correlated with total fatty acid. The vectors indicate the correlation between each biomarker group and the axes. The vetch and vetch plus compost samples, with the lowest values in canonical variate 1, tended to have higher proportions of protozoan and fungal biomarkers than the other samples. While the Gram+ and Gram– bacteria have significant vectors, the vectors are primarily aligned with canonical variate 2, which is much less significant than canonical variate 1. The horizontal separation between the cover-cropped treatments and all other treatments in this graph is primarily due to the protozoan and fungal biomarkers.

## 4. Discussion

Although fertilizer nitrogen rates were lowered to compensate for nitrogen mineralization from the manure, compost, and vetch residue, these treatments still improved yields in 2000, although only when manure and compost were applied at the highest rates tested. This may be because mineralization of manure and compost was enhanced by higher temperatures under black plastic mulch and vetch residue mineralization was enhanced by a consistent, well-distributed rainfall pattern following planting (27.5 mm average rainfall per week during the first 6 weeks).

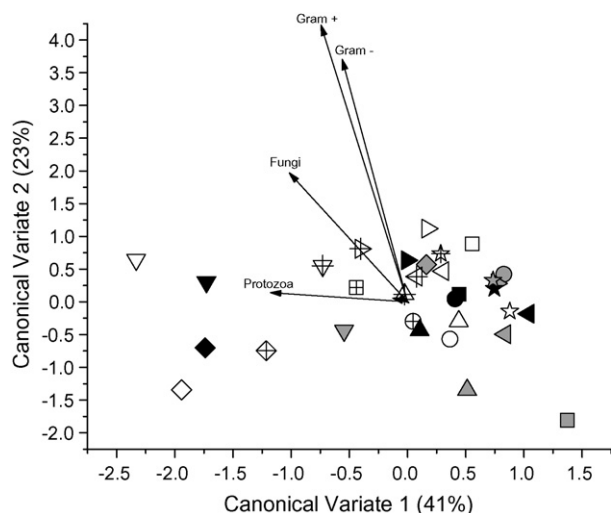
In 2001, yields were lower than in 2000 due to foliar disease. The two highest levels of compost, and the compost plus vetch treatment, had the highest yields. This may be due to higher rates of nitrogen mineralization from these treatments than expected and/or to induced disease resistance that has been observed in compost grown plants (Hoitink et al., 1997). The

**Table 2 – Significance testing results for fatty acid analysis of soil samples**

	Before transformation		After transformation	
	Date <sup>a</sup>	Treatment <sup>b</sup>	Date	Treatment
Chemical group				
Saturated	S	NS	NS	NS
Branched	NS	NS	NS	NS
Monounsaturated	S	NS	NS	NS
Polyunsaturated	S	S	S	S
2-Hydroxy	NS	NS	NS	NS
3-Hydroxy	S	NS	S	NS
Unsaturated branched	S	NS	S	NS
Cyclo	HS	NS	NS	NS
Dimethyl acetal	NS	NS	NS	NS
Overall	S	S	S	S
Biomarker group				
Gram+	NS	NS	NS	NS
Gram–	S	NS	NS	NS
Fungi	S	HS	NS	S
Actinomycetes	NS	NS	S	NS
Mycorrhiza	S	NS	NS	NS
Protozoa	S	S	S	HS
Overall	S	S	S	HS

<sup>a</sup> HS, highly significant ( $P \leq 0.001$ ); S, significant ( $P \leq 0.05$ ); NS, not significant ( $P > 0.05$ ).

<sup>b</sup> All date  $\times$  treatment interactions were not significant.



**Fig. 5 – Canonical variates for fatty acid biomarkers after Hellinger transformation.** Means for each sampling date  $\times$  treatment combination are plotted. Vectors represent standardized canonical coefficients and indicate the relative contribution of each biomarker group to each canonical variate. Several vectors were too short to be plotted, indicating insignificant contributions to the canonical variates. Black symbols, 6/18/01; open symbols, 7/17/01; gray symbols, 8/15/01; crossed symbols, 9/06/01. (■) compost 5 Mg/ha; (●) compost 10 Mg/ha; (▲) compost 20 Mg/ha; (◄) poultry manure 2.5 Mg/ha; (►) poultry manure 5 Mg/ha; (★) fertilizer; (◆) hairy vetch; (▼) vetch plus compost 10 Mg/ha.

relatively low yield of the vetch treatment, when compared to the two highest level of compost, was probably the result of buildup of weed populations, particularly yellow nutsedge (*Cyperus esculentus* L.), in this treatment. The vetch treatments were without a covering of black plastic mulch to suppress weeds but relied on the vetch residue and herbicides to control weeds. Yellow nutsedge is tolerant of surface residue and of the herbicides used in these plots (metribuzin and napropamide) and became particularly troublesome during the wet conditions encountered in 2001, conditions under which this weed thrives. The vetch plus compost treatment was less affected by weeds than the vetch-only treatment because the vetch plus compost treatment was only established in 2001 and there was insufficient opportunity for the buildup of nutsedge populations during the previous year as occurred in the hairy vetch treatment.

We hypothesized that hairy vetch cover cropping would increase crop yield over the plastic mulch and synthetic fertilizer system. While there was a statistically insignificant increase in 2000, there was no increase at all in 2001. Yield was approximately 18% higher in hairy vetch than the fertilizer-based system in 2000, an increase comparable to that seen in previous research (Abdul-Baki and Teasdale, 1997), but this difference was not significant ( $P = 0.05$ ). There was no difference between these systems in 2001, probably because of unanticipated weed populations in vetch in that year. We further hypothesized that organic amendments, when used

with plastic mulch, would increase yields over synthetic fertilizer and plastic mulch systems. This was found to be true in both years, although the relative performance of manure and compost varied from year to year. More research is needed to understand the influence of plasticulture on mineralization of N from soil amendments.

Sampling date was clearly more significant than treatment in determining the community structure of rapidly growing, culturable, aerobic, heterotrophic bacteria, as measured using Biolog GN plates. Separation by date was primarily due to an increase in overall absorbance, which was probably caused by an increase in inoculum density. However, even after the data was transformed, there was still some date effect. Since the transformation removed any effect of overall absorbance there must have been some date effect on community structure as well as on population density. The treatment effect observed in the canonical variates analysis was due to differences in community structure between the two vetch treatments and all other treatments. This was consistent with our first hypothesis, that hairy vetch cover cropping would affect soil microbial community structure, but was inconsistent with our second and third hypotheses.

Soil microbial community structure, as measured by fatty acid analysis, was affected by both date and treatment. Hairy vetch cover cropping, particularly when combined with compost amendment, was associated with an increase in the relative amount of polyunsaturated fatty acids, including the biomarkers for fungi and protozoans. This was consistent with hypothesis 1, but inconsistent with hypotheses 2 and 3. A previous study on vegetable cropping systems also found that fungal and protozoan fatty acid biomarkers were increased by cover cropping (Schutter et al., 2001). While the fungal biomarker fatty acid 18:2  $\omega$ 6c also occurs in higher plants, we do not believe that the treatment effect observed was due to input of fatty acids from hairy vetch because there was no overall increase in total fatty acids in the vetch treatments compared to the other treatments. Also, if vetch fatty acids were entirely responsible for the treatment effect, then one would predict that vetch and vetch plus compost treatments would behave similarly, and there clearly are some differences between them in the canonical variates analyses. The increase in fungal biomarker fatty acid with cover cropping was not, however, reflected in an increase in fungal substrate utilization as expected. There may have been a significant fungal population that did not grow in the Biolog SF-N plates.

Previous studies have found that amendment with farm-yard manure (Toyota and Kuninaga, 2006), grape compost (Saison et al., 2006), and spent mushroom compost (Pérez-Piqueres et al., 2006) significantly affected soil microbial community structure. However, the effects of compost were found to vary depending on both the type of compost and the soil type (Pérez-Piqueres et al., 2006). The effects of sewage sludge, turkey manure, and composted turkey manure were found to vary with the specific amendment and were transient (Calbrix et al., 2007). It may be that we did not see significant effects of manure or compost because of the specific compost we used, the short-term nature of the study, or the soil type. On-farm research revealed that annual or biennial applications of dairy manure resulted in soil microbial communities distinguishable from soil that received no

manure or occasional applications (Bucher and Lanyon, 2005), suggesting that we might have detected a compost or manure effect if our study had continued for several more years.

The community structure of rapidly growing, culturable, aerobic, heterotrophic bacteria was affected by cover cropping as compared to plastic mulch, although fatty acid analysis did not indicate any treatment effect on the total soil bacterial community. The copiotrophic, or “r”-selected bacteria, that are detected in the substrate utilization assay, would be expected to be most rapidly affected by the high level of organic carbon and nitrogen found in root exudates and plant residue produced by the cover crop, while the oligotrophic or “K”-selected bacteria that are undetectable in the substrate utilization assay would be expected to respond much less, and much more slowly, to cover crop nutrient input. Since the vast majority of soil bacteria are uncultured, and may well be oligotrophic (Watve et al., 2000), the fatty acid analysis for total soil bacterial community structure might be expected to show less treatment effect than the substrate utilization assay for bacteria, which is what we found. This is consistent with earlier results on spermosphere and rhizosphere communities (Bååth et al., 1998; Buyer et al., 1999, 2002).

While the most obvious differences between the hairy vetch and plastic mulch treatments are the input of exudates and plant residue from the cover crop, there are other possible explanations as well, including soil temperature. Hairy vetch mulch has been shown to decrease soil temperature compared to black polyethylene (Teasdale and Abdul-Baki, 1995). In culture, lower incubation temperatures are associated with increases in unsaturated fatty acids, short-chain fatty acids, and branched fatty acids (Lechevalier and Lechevalier, 1988). The increase in polyunsaturated fatty acids that we observed in the vetch treatments may therefore be in part an artifact of lower soil temperature rather than a change in microbial community structure. However, there was no increase in monounsaturated or branched fatty acids as would be expected. Also, this argument does not explain the treatment effect observed in the bacterial substrate utilization assay (Fig. 2a and b). It is clear that hairy vetch cover cropping resulted in higher levels of biomarker fatty acids for protozoans and fungi, which presumably was correlated with higher population densities of protozoans and fungi, and also affected the community structure of fast-growing, aerobic, heterotrophic bacteria.

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